Predation-induced plasticity in metamorphic duration in Xenopus laevis

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Predation-induced plasticity in metamorphic duration in *Xenopus laevis*

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Summary

1. Many organisms are able to vary the duration of life-history stages in response to environmental conditions such as predation risk. However, in those undergoing a metamorphosis, it is not known whether the duration of the metamorphic phase itself can change in response to the presence of a predator, and whether this carries costs.

2. In experiments using the amphibian *Xenopus laevis*, we found that metamorphosis was accelerated in the presence of a predator and this occurred consistently across the natural range of temperatures experienced by *Xenopus*.

3. Although metamorphic climax was reduced in duration, a functional tail was maintained for longer in the presence of a predator. Furthermore, burst swimming speed was significantly faster for animals metamorphosing in the presence than in the absence of a predator. This suggests that the more rapid development induced by predator presence does not carry costs in terms of ability to escape predators during metamorphosis.

4. There was no evidence of post-metamorphic costs of faster metamorphic climax in terms of escape response since juveniles from the two predator treatments did not differ in burst swimming speed. However, individuals metamorphosing in the presence of predators lost proportionally more mass during metamorphosis, resulting in smaller juveniles than those without predators. This reduced juvenile size represents a potential cost of accelerating metamorphic development.

5. Therefore, conditions experienced during metamorphic climax, independent of larval conditions, can have a significant influence on the life histories of animals with complex life cycles.

Key-words: life-history variation, locomotor performance, metamorphic climax, predation, *Xenopus laevis*

Introduction

In organisms with complex life cycles, that is with distinct life-history stages and marked between-stage transitions during which the phenotype is re-shaped, environmentally induced plasticity in stage duration and morphology have been well studied (West-Eberhard 2003). Predation risk has been identified as an important environmental factor, being linked to the development of morphological and behavioural anti-predator defences and changes in the duration of life-history stages (reviewed in Benard 2004; Relyea 2007). The main focus of these studies has been on the effects of predation risk during embryonic and larval life-history stages (Nylin & Gotthard 1998; Benard 2004). However, it is also likely that predation risk will influence the duration of the transition stages, especially where such stages might render individuals more vulnerable to predators, for example, if they are less active or mobile.

In most amphibians and insects, metamorphic climax, the beginning of which marks the end of the larval phase and its conclusion the formation of the juvenile (or adult) in most species, represents a clearly defined transition stage (Bishop *et al.* 2006). During metamorphic climax, individuals are generally more vulnerable, due to reduced locomotor performance, and are captured more frequently by predators (Wassersug & Sperry 1977; Arnold & Wassersug 1978). Predation risk experienced throughout larval and metamorphic stages has recently been shown to affect the duration of metamorphosis in insects (McKie & Pearson 2006), while Van Buskirk & Saxer (2001) did not find an effect of predator-induced larval phenotypes on the duration of metamorphic climax in amphibians. However, the effects of predation-risk experienced during metamorphic climax on its duration have not been assessed independently of predation-induced effects

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on the larval phenotype, nor have potential costs of accelerated metamorphosis been identified.

During metamorphic climax, the developmental response to predation risk might be similar to that observed during embryonic development, that is, acceleration under heightened predation risk (Martin 1995; Warkentin 1995; Chivers *et al.* 2001; Li 2002; Kusch & Chivers 2004; Vonesh 2005), since both are non-feeding stages with reduced locomotor ability compared to the subsequent life stage. However, more than one environmental factor is likely to be important, and there may be interactions among different factors acting simultaneously. Temperature is known to be an important variable affecting the rate of development (Gillooly *et al.* 2002). The duration of metamorphic climax has been found to change with environmental temperature in amphibians and insects (Downie, Bryce & Smith 2004; Stevens 2004; Walsh, Downie & Monaghan 2008a). The observed acceleration of development with increasing temperature is likely to level off at higher temperatures since the capacity to accelerate development at higher temperatures is limited either due to prohibitive costs or a biological constraint (Bottrell 1975; Wagner *et al.* 1984; Li & Jackson 1996; Roy, Brodeur & Cloutier 2002; Downie *et al.* 2004; Walsh *et al.* 2008a; Walsh, Downie & Monaghan 2008b). Hence, we would expect that the acceleration of metamorphic climax in response to predation risk will be most marked at lower temperatures.

In amphibians, it has also been shown that, in the presence of predators, tadpoles alter their tail morphology to aid predator evasion (e.g. McCollum & Van Buskirk 1996; Wilson, Kraft & Van Damme 2005; Benard 2006). However, it is not known whether morphology expressed during metamorphic climax responds to predation-risk. Greater tail length and depth have both been shown to improve locomotor performance during larval development (Dayton *et al*. 2005; Teplitsky *et al*. 2005; Kaplan & Phillips 2006). Therefore, in the presence of predators, metamorphosing individuals might delay the beginning of rapid tail re-absorption, thus retaining a functional tail for a longer period during metamorphosis to aid in locomotion until the hind limbs are better developed (Wassersug & Sperry 1977; Huey 1980).

In this study, we examined the plasticity of metamorphic duration and tail re-absorption during metamorphosis in the fully aquatic amphibian *Xenopus laevis* in relation to predator presence and variation in environmental temperature only during metamorphosis. While *Xenopus* does not leave the aquatic environment, it does undergo an extensive hormonally induced metamorphosis involving alteration of every major organ system including changes in head morphology, remodelling of the gastro-intestinal tract and complete absorption of the tail (Furlow & Neff 2006). This last effect results in a transition from tail driven locomotion in the tadpole to limb-propelled locomotion in the juvenile (Combes *et al.* 2004) resulting in a rapid increase in burst swimming performance upon completion of metamorphosis (Walsh *et al.* 2008a). *Xenopus* also transforms from an herbivorous forager in the water column to a camouflaged, largely bottom-dwelling ambush predator on completion of metamorphosis (Tinsley & Kobel 1996). As a result of the changes in speed and niche, the relative predation risk of *Xenopus* is likely to differ during ontogeny. Additionally, since *Xenopus* remains in the aquatic environment, locomotor performance at different stages can be compared directly. Finally, a fully aquatic life cycle enables the potential inability to perceive altered mortality-risk in a different habitat to be removed as a confounding factor in comparisons between life stages (Benard 2004). Locomotor performance and body size during metamorphosis and following its completion were assessed in order to identify possible costs associated with predator-induced changes in metamorphosis.

Methods

ANIMALS AND REARING CONDITIONS

Approximately 250 *X. laevis* eggs were obtained from St Andrews University, Scotland in 2006. Tadpoles were kept in a single 40-L holding tank at 24 °C until approximately Nieuwkoop & Faber (NF) stage 42 (Nieuwkoop & Faber 1994) before being transferred to 10 smaller holding tanks.

Each smaller holding tank (30 × 20 × 20 cm) contained 25 tadpoles in *c.* 11 L of aerated, de-chlorinated, copper-free water. Water temperature in the tanks ranged from 30 °C (daytime) to 18 °C (night), which represents the natural range of temperatures experienced by *X. laevis* (Tinsley & Kobel 1996). Tadpoles were exposed to a 12L : 12D photoperiod and fed *ad libitum* on algal pellets, ground and hydrated before being suspended in water. Checks were made three times a day for individuals commencing metamorphic climax, indicated by the emergence of one or both of the fore limbs and coinciding with a surge in thyroid hormone (Shi 2000) (NF stage 60; equating to Gosner (1960) stage 42); this stage is recognised as the end of the larval phase (e.g. Hensley 1993). The conclusion of metamorphic climax was identified by the complete absorption of the tail (tail length < 0·5 mm, NF stage 66; equating to Gosner stage 46).

EXPERIMENTAL DESIGN

A 2 × 3 factorial experimental design was used to examine the effects of predator risk and temperature on the duration of metamorphic climax, morphology and locomotory performance during and after the completion of metamorphosis. To evaluate the effects of predator presence, with and without predator treatments were used. Adult *X. laevis* (SVL: 4–6 cm) were used as predators since adults eat larvae in this species, both in the laboratory and in the field (Hey 1946; Tinsley & Kobel 1996). All experimental tanks had clear, perforated Perspex dividing them into two equal segments (15 × 20 × 20 cm). An adult was placed on one side of the Perspex divide in half the experimental tanks and fed on a diet of bloodworms (Family: Chironomidae) and *X. laevis* tadpoles (frozen prior to first feeding NF stage 38) to ensure that any predation-associated chemical cues were present in the water (Petranka, Kats & Sih 1987). Three constant temperature treatments were established; 18 °C, 24 °C and 30 °C.

In total, 163 tadpoles were transferred from the small holding tanks to individual experimental tanks at the onset of metamorphosis. The 18 °C and 24 °C treatments had 54 individuals, while the 30 °C had 55. The remainder were not included due to natural larval mortality or failure to commence metamorphosis during the experimental period.

Wet mass after removal of surface water (0·001 g), snout-vent length (SVL), head width, tail length and maximum tail depth measurements (0·1 mm, using calipers) were taken at the commencement of metamorphic climax; anaesthetising the tadpoles for this, which carries a significant mortality risk, was deemed unnecessary since the tadpoles remained quiescent and were out of the water for a very brief period. There were no significant differences in mass (942 ± 26 mg), SVL (19·5 ± 0·2 mm), head width (9·6 ± 0·1 mm), tail length (36·8 ± 0·4 mm) or tail depth (7·1 ± 0·1 mm) at the start of metamorphosis among the six treatments.

Tail length and depth measurements were taken every 3 days during metamorphosis. The number of days that a functional tail length and depth was retained was taken as the time until 50% of the tail was absorbed (Van Buskirk & McCollum 2000). This was calculated from the sigmoid regression equation, solving for x ($x = b(\ln(a - y/y)) - x_0$, where y = 50%, b is steepness of the curve, a is the maximum asymptote and x_0 is the inflection point).

Individuals were checked three times a day for completion of metamorphic climax, at which point mass, SVL and head width were measured. The number of days between the onset of metamorphic climax and its completion was also recorded. Post-metamorphic individuals were maintained at their experimental temperature until testing then transferred to a stock tank maintained at 24 °C. Only six mortalities occurred during metamorphosis; in the presence of a predator all three temperature treatments experienced a single mortality; at 30 °C the 'no predator' treatment had three mortalities.

SWIMMING SPEED DATA COLLECTION

Locomotor performance was measured halfway through metamorphosis (NF stage 63) and after metamorphosis was complete (NF stage 66+) on a randomly selected sample of individuals, using a Photron FASTCAM-PCI high-speed camera. From each treatment, 15 individuals were assessed for locomotor performance, with the exception of the no predator 18 °C treatment where 16 individuals were assessed. One of the 'predator-present' 30 °C treatment individuals that was assessed during metamorphosis died, so only 14 juveniles in that treatment group were assessed for locomotor performance. Due to the sensitivity of the equipment, all filming was performed in one room. Prior to filming, individuals were acclimatized to the room temperature (19–22 °C) for 1 h. Slight variation in room temperature at the time of filming, which was included as a covariate, did not affect locomotion in metamorphs or juveniles (Table 2). The 1-h time period was selected as an acceptable period for acclimatization, as a longer period may have affected the developmental processes being investigated. Filming was done at 250 frames per second (fps). Each individual was filmed six times. There was no evidence of habituation in either the metamorphic ($F_{5,335}$ = 0·879, P = 0·495) or juvenile individuals ($F_{5,315}$ = 1·625, P = 0·153).

The camera was placed directly above a 20 × 4 cm tank, lined with laminated grid-paper filled with *c.* 1 cm depth of water. Each individual was placed at one end of the tank, and then gently prodded at the base of the tail to elicit an escape response. The tip of the snout was tracked over successive frames. Linear distance travelled per frame, following initial movement, was measured to give an average velocity (cm s^{-1}) over the first 37 frames (294 ms), using Photron Motion Tools software, which generally represents the burst speed critical in fleeing from a predator (Walker *et al.* 2005; Wilson *et al.* 2005; Royle, Metcalfe & Lindstrom 2006). The results using the maximum burst speed from the six trials are reported, since it represents the fastest burst speed of which an individual was capable.

STATISTICAL ANALYSIS

All analysis was performed using SPSS v15 (SPSS Inc., Chicago, IL) unless otherwise stated. Metamorphic duration and swim speed analyses were corrected for body size. SVL (accounting for 84·4% of the variation using principal components analysis of mass, SVL, head width, tail length and tail depth) was used as an indicator of body size since all structural body and tail measurements taken were highly correlated. Metamorphic duration was examined using a General Linear Mixed Model (GLMM), with the holding tank that the tadpole originated from as a random factor. The holding tank (P = 0·45) and interaction term (P = 0·22) were not significant and were removed to give the minimum adequate model. The time to lose 50% of the tail depth was determined using 3-parameter, sigmoid regression (SigmaPlot 10, ystat Software Inc., Chicago, IL) and analysed using a GLM. Since the re-absorption of tail length followed the same pattern as tail depth re-absorption in relation to predator presence and temperature, tail length results are not presented here. Remaining analyses were performed using GLM, non-significant interaction terms were removed. In analysis of locomotor performance, SVL and tail depth at the time the trials were performed were also included as covariates. Additionally, locomotor performance was analyzed without SVL as a covariate, to investigate the treatment effects on actual swimming performance. The overall results of the model were the same, so only size-corrected results are reported.

Results

METAMORPHIC DURATION

After correcting for body size (SVL) at the start of metamorphic climax ($F_{1,156}$ = 64·48, P < 0·0001), the presence of a predator resulted in a reduction in metamorphic duration of between 4% and 7% across the three temperature groups ($F_{1,156}$ = 10·13, P = 0·002; Fig. 1). Duration was longest in the 18 °C temperature treatment, followed by the 24 °C and 30 °C treatments ($F_{2,156}$ = 1819·43, P < 0·0001).

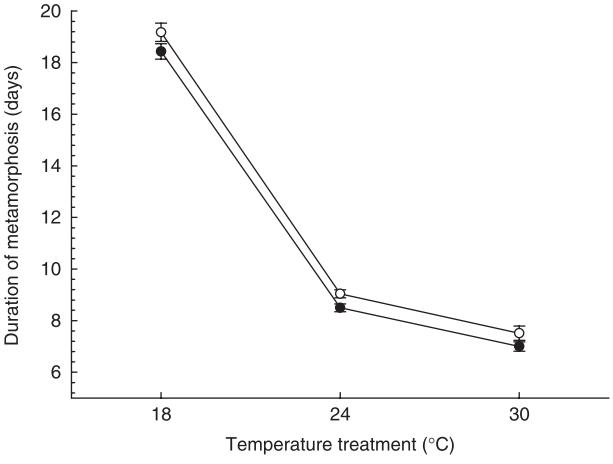

Fig. 1. Duration of metamorphic climax in days (± SE) for the three temperature treatments and the presence or absence of a predator. Metamorphic climax begins with the emergence of the fore limbs, which coincides with a peak in thyroid hormones, and is concluded with complete tail absorption. Open shapes represent the no predator treatment and solid shapes represent the predation treatment.

Table 1. Results of GLM analyses for the juvenile mass and structural size measurements of individuals that completed metamorphosis in the presence or absence of a predator and among the three temperature treatments. For each analysis, the measurement at the start of metamorphosis was included as a covariate. The temperature × predator treatment interaction term ($P > 0{\cdot}27$) and the holding tank that individuals originated from ($P > 0{\cdot}38$) were not significant in any of the analyses and are not included

Dependent variable	Predator treatment			Temperature treatment		
	df	*F*	*P*	df	*F*	*P*
Mass	1,156	3·95	0·049	2,156	14·98	< 0·0001
Snout-vent length	1,156	11·95	< 0·0001	2,156	10·18	< 0·0001
Head width	1,156	7·70	0·006	2,156	5·32	0·006

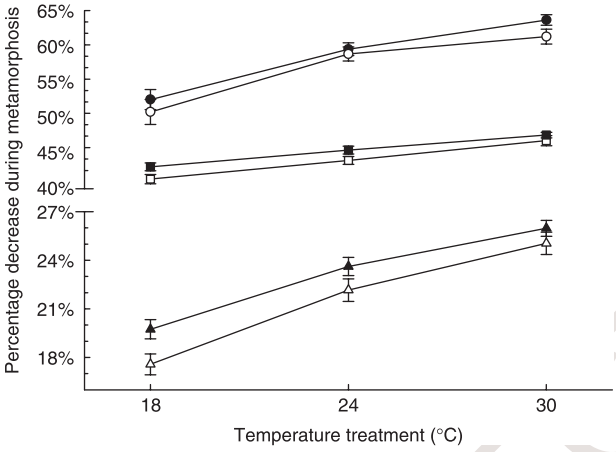

Fig. 2. Mean percentage loss of mass (●), SVL (▲) and head width (■) during metamorphosis for each temperature and predator treatment (± SE). Open shapes represent the no predator treatment and solid shapes represent the predation treatment.

Despite the shorter duration of metamorphosis, individuals that completed metamorphosis in the presence of a predator lost more mass and structural body size in relation to their size at the start of metamorphic climax than those in the no predator treatment (Table 1, Fig. 2). This was again consistent across the temperature treatments. In line with this, the shorter metamorphic duration at higher temperatures was also associated with smaller post-metamorphic body mass and size (Figs 1 and 2).

FUNCTIONAL TAIL RETENTION

Tail re-absorption, shown here as tail depth (length followed the same pattern), followed a sigmoid pattern (Fig. 3). The length of time for which a functional tail was retained, measured as the time taken to absorb 50% of the tail depth, was greater in the presence of a predator than when there was no predator ($F_{1,148} = 5{\cdot}86$, $P = 0{\cdot}017$). Low temperature also extended the period for which a functional tail depth was retained ($F_{2,148} = 655{\cdot}56$, $P < 0{\cdot}0001$).

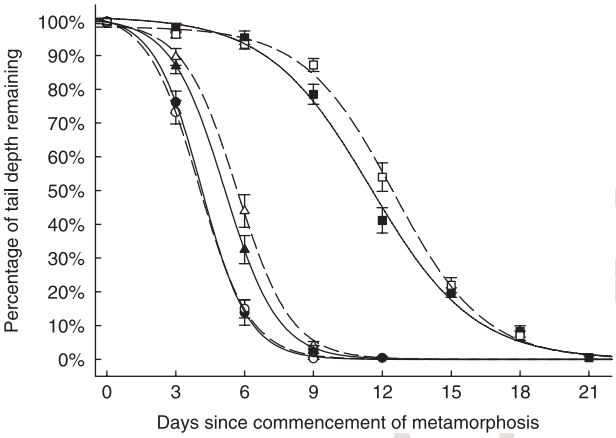

Fig. 3. Percentage decrease in mean tail depth (± SE) through metamorphosis (■: 18 °C, ▲: 24 °C, ●: 30 °C. For all three temperature treatments, the predator absent treatment is indicated by filled shapes and solid regression lines, while the predator present treatment is indicated by open shapes and broken regression lines).

Table 2. Results of GLM analysis of locomotor performance of individuals at mid-metamorphosis and at the completion of metamorphosis in relation to predator and temperature treatments. The interaction between temperature and predator treatment were non-significant and removed ($P > 0{\cdot}37$)

	Metamorphosing individuals			Post-metamorphic individuals		
	df	*F*	*P*	df	*F*	*P*
Temperature	2,91	1·41	0·25	2,90	1·88	0·16
Predator	1,91	6·23	< 0·02	1,90	0·44	0·51
SVL	1,91	30·41	< 0·0001	1,90	87·39	< 0·0001
Tail depth	1,91	1·46	0·23	–	–	–
Predator × SVL	2,91	7·64	< 0·01	2,90	0·71	0·40
Room temperature	1,91	0·33	0·57	1,90	1·95	0·17

LOCOMOTOR PERFORMANCE

During metamorphosis (NF stage 63), independent of temperature treatment and after correcting for body size (see Methods), those tadpoles metamorphosing in the presence of a predator showed maximum swimming speeds *c.* 5% faster compared with the no-predator treatment (Table 2, Fig. 4). Independent of the significant effect of body size, tail depth at the time of the trials was not found to significantly affect swimming speed during metamorphosis (Table 2). There was also a significant interaction between predator treatment and SVL (Table 2). In the predator treatment, burst swim speed increased faster with increasing SVL than in the no predator treatment.

On completion of metamorphosis (NF stage 66), mean maximum swim speed was 34·71 cm s^{-1} ± 0·89 SE (2·26 body length s^{-1} ± 0·04 SE). There was no significant difference between predator and no predator treatments, independent of experimental temperature treatment, when corrected for the significant effect of SVL (Table 2).

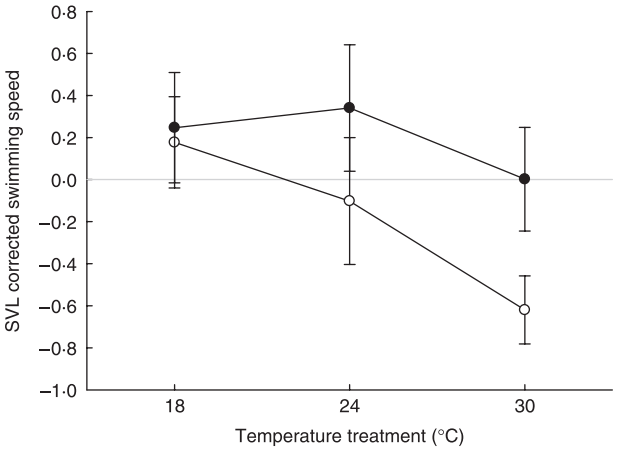

Fig. 4. Maximum burst speed (± SE) of metamorphosing (NF stage 63) individuals (represented as the residuals corrected for SVL) that are metamorphosing in either the presence or absence of a predator at each of the three temperature treatments. Open circles represent the no predator treatment and solid circles represent the predation treatment.

Discussion

In *X. laevis* both duration of metamorphic climax and tail morphology during metamorphosis were found to vary, as predicted, in response to predator presence. This demonstrates for the first time that increased predation risk experienced only during metamorphic climax induces variation in the duration of this transition stage. The presence of a predator during metamorphosis resulted in shorter duration of metamorphosis at all three temperature treatments, with no difference in the degree of acceleration observed among the temperature treatments. Thus there was no interaction between the predator presence and temperature across the temperature range used in this study, possibly because the range used did not extend to the temperature threshold where acceleration of development is constrained (McKie & Pearson 2006).

It has been proposed that selection will favour shortening the duration of life-history stages, such as amphibian metamorphosis, with heightened vulnerability to predation, since this is likely to confer significant fitness benefits (Williams 1966; Istock 1967; Wassersug & Sperry 1977; Arnold & Wassersug 1978; Rose 2005). That metamorphosis is longer in the absence of predators suggests that shortening is sufficiently costly to favour retention of the capacity to adjust metamorphic duration in response to local predation risk. In the absence of predators, it pays to take longer over metamorphosis. One potential cost associated with rapid growth or development is an impairment of locomotor performance in subsequent stages (Kolok & Oris 1995; Arendt 2003; Buckley, Michael & Irschick 2005; Ficetola & De Bernardi 2006; Ji *et al*. 2006). However, we did not find any difference in post-metamorphic locomotion amongst our treatment groups. Similarly, Capellan & Nicieza (2007) and Van Buskirk & Saxer (2001) did not find a cost in terms of locomotor performance due to predator presence during the hatching or larval stages, respectively, in the juvenile stage of ranid species. Therefore, it appears that the acceleration of metamorphosis in *X. laevis* does not carry associated locomotor costs, measured in burst swim speed, as in *Rana temporaria* (Capellan & Nicieza 2007) and *R. ridibunda* (Van Buskirk & Saxer 2001). However, costs might become apparent later in life (Arendt 1997; Metcalfe & Monaghan 2001; Morgan & Metcalfe 2001) or be evident in other fitness components or measures (e.g. sustained locomotor performance).

Metamorphosing individuals that experienced the presence of a predator showed a greater decrease in mass, SVL and head width than those that metamorphosed in the absence of a predator. Although this did not directly result in reduced absolute or size-corrected burst swim speed, a reduction in size on the completion of metamorphosis, in amphibians, has been shown to reduce survival, size at first reproductive bout and reproductive output, and increase the age at first reproduction (Collins 1979; Berven & Gill 1983; Smith 1987; Semlitsch, Scott & Pechmann 1988; Berven 1990; Scott 1994; Beck & Congdon 2000; Altwegg & Reyer 2003; Chelgren *et al.* 2006).

Even though metamorphosis was accelerated in the presence of a predator, a functional tail was retained for longer, but more rapidly re-absorbed towards the end of metamorphosis. Given the shorter metamorphic durations experienced in the presence of a predator, the increased time period of functional tail retention would mean that in the presence of a predator individuals retain over 50% of their tail depth for a substantially greater proportion of metamorphosis. Retention of tail depth would be expected to benefit locomotion at this stage; however this was not found to be the case, at least with respect to burst swim speed. While locomotor performance increased under predation risk during metamorphosis, this was not related to variation in tail depth (or length). This could be due to several reasons. Tail retention might only have an effect on locomotor performance at earlier stages, prior to when burst speed was assessed in this study, or in more sustained swimming. The faster swimming speeds observed in the predator treatment could be the result of factors other than tail depth (or length) (Van Buskirk & McCollum 2000), such as accelerating the development of functional hind limbs to aid in locomotion. Conversely, the predator treatment individuals may have a heightened sensitivity or alertness to a potential predator stimulus, due to exposure to predation-associated chemical cues (Pfeiffer & Riegelbauer 1978; Hews & Blaustein 1985; Hews 1988).

In conclusion, our data demonstrate that during metamorphosis *X. laevis* shows plasticity in both morphology and duration in response to predator presence. This provides further evidence that metamorphosis itself, which has been largely overlooked in studies of phenotypic plasticity, is not fixed in duration and that the variability in responses to environmental conditions observed during larval development may be expected during metamorphosis. Thus, the habitat experienced during metamorphosis may have important implications for the pattern and tempo of phenotypic development.

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